

## Interactions of uranium with bacteria and kaolinite clay

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### Abstract

We assessed the accumulation of uranium (VI) by a bacterium, *Bacillus subtilis*, suspended in a slurry of kaolinite clay, to elucidate the role of microbes on the mobility of U(VI). Various mixtures of bacteria and the kaolinite were exposed to solutions of  $8 \times 10^{-6}$  M- and  $4 \times 10^{-4}$  M-U(VI) in 0.01 M NaCl at pH 4.7. After 48 h, the mixtures were separated from the solutions by centrifugation, and treated with a 1 M CH<sub>3</sub>COOK for 24 h to determine the associations of U within the mixture. The mixture exposed to  $4 \times 10^{-4}$  M U was analyzed by transmission electron microscope (TEM) equipped with EDS. The accumulation of U by the mixture increased with an increase in the amount of *B. subtilis* cells present at both U concentrations. Treatment of kaolinite with CH<sub>3</sub>COOK, removed approximately 80% of the associated uranium. However, in the presence of *B. subtilis* the amount of U removed was much less. TEM–EDS analysis confirmed that most of the U removed from solution was associated with *B. subtilis*. XANES analysis of the oxidation state of uranium associated with *B. subtilis*, kaolinite, and with the mixture containing both revealed that it was present as U(VI). These results suggest that the bacteria have a higher affinity for U than the kaolinite clay mineral under the experimental conditions tested, and that they can immobilize significant amount of uranium. © 2005 Elsevier B.V. All rights reserved.

**Keywords:** Uranium; *Bacillus subtilis*; Kaolinite; Accumulation; Bacteria–mineral mixture; Migration

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### 1. Introduction

The migration of uranium from uranium-mining operations and the disposal of radioactive wastes are major environmental concerns (Buck et al., 1996; Airey and Ivanovich, 1986). Uranium typically occurs

as hexavalent uranyl aqueous complexes in oxic environments (Langmuir, 1978). The mobility of U(VI) is determined by its interactions with soils and subsoils composed of abiotic and biotic components, principally minerals and bacteria, respectively (Dent et al., 1992; Ticknor, 1994; Waite et al., 1994; Sylwester et al., 2000; Fowle et al., 2000; Haas et al., 2001; Francis et al., 2004). There have been extensive studies done on the accumulation of U(VI) by bacteria (Lovley et al., 1991; Fowle et al., 2000; Haas et al., 2001; Brantley et al., 2001; Francis et al., 2004) and by minerals (Dent et al., 1992; Ticknor, 1994; Waite et al., 1994; Sylwester et al., 2000). However, as far as we are aware, little is known about U sorption in a mixture of bacteria and minerals.

Studies of U(VI) interactions with bacteria showed that U(VI) may be associated with the functional groups on the cellular surface (Fowle et al., 2000; Haas et al., 2001; Francis et al., 2004), precipitated to form uranyl-containing minerals (Macaskie et al., 1992; Young and Macaskie, 1995; Jeong et al., 1997), or reduced to insoluble U(IV) (Lovley et al., 1991; Francis et al., 1994; Suzuki et al., 2002). Studies with aluminosilicates minerals revealed that U(VI) is absorbed by the reactive groups of the minerals at pHs between 3 and 5. Therefore, an understanding of the behavior of U(VI) in soils and rocks requires a detailed knowledge of its interactions not only with the bacterial and mineral surfaces, but also within a mixture of bacteria and minerals.

In this study we investigated the accumulation of U by mixtures of *Bacillus subtilis* and kaolinite. *B. subtilis* and kaolinite were chosen because (a) both are ubiquitous in the terrestrial environment; and, (b) their surfaces are well characterized. We also determined whether U showed preferential affinity to bacteria, kaolinite clay or in a mixture of both. In addition to carrying out sorption and desorption experiments, we examined the association of U by transmission electron microscopy (TEM).

## 2. Materials and methods

### 2.1. Microorganism, kaolinite, and U solution

*B. subtilis* (IAM 1069) was obtained from the Institute of Molecular and Cellular Biosciences, The

University of Tokyo. This strain is a Gram-positive, rod-shaped heterotrophic bacterium. The cells were grown for 40–48 h in 500-mL conical flasks at 30 °C in 250 mL sterilized liquid growth medium containing meat extract (3 g L<sup>-1</sup>), polypeptone (5 g L<sup>-1</sup>), and NaCl (5 g L<sup>-1</sup>). Cells at the stationary growth phase were harvested by centrifugation at 2500×g for 10 min, and washed twice by 0.1 M NaCl. They finally were resuspended in a 0.01 M NaCl solution and immediately used in the experiments. An aliquot of the cell suspension was centrifuged and dried overnight at 70 °C to determine the dry weight of the cells in the stock suspension.

Commercial kaolinite from Nihon Chikagaku-sha Co. Ltd., Kyoto, Japan was used. Kaolinite was the only mineral identified by X-ray powder diffraction. The specific surface area of the kaolinite was 26.4 m<sup>2</sup> g<sup>-1</sup>, assessed by the Brunauer Emmett Teller (BET) method. The kaolinite was washed twice with distilled deionized water and suspended as a slurry to a concentration of 100 g L<sup>-1</sup>.

Natural U stock solutions of different U concentrations were prepared by dissolving UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O in 0.01 M NaCl. The <sup>233</sup>U isotope was added to the natural U stock solutions to achieve the activity required for radiometric measurements.

### 2.2. Accumulation experiments

The accumulation of U(VI) by biotic and/or abiotic components depends on the chemical species of U(VI). At pHs between 3 and 5, U(VI) is present predominantly as dissolved U(VI)O<sub>2</sub><sup>2+</sup> species (Suzuki and Banfield, 1999). In solution above pH 7, its major chemical species are uranyl carbonate complexes; a small portion of U(VI) can be precipitated to form uranyl hydroxides (Langmuir, 1978). We choose a solution at pH 4.7 to avoid the precipitation of U(VI) hydroxide and formation of U(VI) carbonate complex species.

To explore the accumulation of U(VI) in mixtures of *B. subtilis* and kaolinite, we exposed mixtures containing 38 g L<sup>-1</sup> kaolinite and 0, 0.19 (0.5% in dry weight percent fraction), 0.38 (1%), 2.0 (5%), or 4.2 g L<sup>-1</sup> (10%) of *B. subtilis* to an 8 × 10<sup>-6</sup> M U(VI) solution at pH 4.7 ± 0.1 for 48 h. The mixtures containing 38 g L<sup>-1</sup> kaolinite and 0, 0.04 (0.1%), 0.19 (0.5%), 0.38 (1%), 1.2 (3%), or 2.0 g L<sup>-1</sup> (5%)

of *B. subtilis* were contacted with a  $4 \times 10^{-4}$  M U(VI) solution for 48 h at pH  $4.7 \pm 0.1$ . Samples containing  $4.2 \text{ g L}^{-1}$  *B. subtilis* but no kaolinite or kaolinite without the bacteria were similarly exposed to the two U(VI) solutions. The pH of the solutions was adjusted by adding 1 M NaOH and 1 M HCl solutions at the start of the experiments, and was readjusted at 2, 18, and 26 h later. The final pH was  $4.7 \pm 0.2$ . The ionic strength of the U solution was adjusted to 0.01 M with NaCl. Duplicate sets of accumulation experiments were carried out at 25 °C in polypropylene centrifuge tubes which have been previously washed with a 0.1 M HClO<sub>4</sub> solution and rinsed with deionized water. At the end of each experiment, 5-mL supernatant was removed for measuring U concentration and pH.

Forty-eight hours after exposure to U solutions, the bacteria and kaolinite mixtures were separated from the solutions by centrifugation at 6000 rpm for 10 min and washed repeatedly with deionized water to remove any U-bearing solution retained through surface tension. Note that U removal by washing with deionized water was less than 3% of the total U. Then, a 1 M CH<sub>3</sub>COOK solution at pH 4.7 was added to each of them, and they were left for 8 h.

After centrifuging the CH<sub>3</sub>COOK solution at 6000 rpm for 10 min to separate them from the mixtures, the concentrations of U in the CH<sub>3</sub>COOK solutions were measured with a liquid-scintillation analyzer with alpha/beta discrimination (Packard Tri-Carb 2550TR/AB) using liquid-scintillation cocktails (Packard Ultima-Gold AB and F). The desorbed fraction of U was normalized to 100 when all of the sorbed U was removed with the CH<sub>3</sub>COOK. A TOA HM-30S pH meter with a combined electrode of TOA GS-5015C was used to measure the pH of the solutions.

The oxidation states of U accumulated on *B. subtilis*, kaolinite in the mixture containing  $2.0 \text{ g L}^{-1}$  *B. subtilis* and  $38 \text{ g L}^{-1}$  kaolinite exposed to a  $4 \times 10^{-4}$  M U(VI) solution at pH 4.7 were analyzed by X-ray absorption near edge structure (XANES). The U L<sub>3</sub>-edge (17166 eV) spectra of powder samples of uraninite (UO<sub>2</sub>) and UO<sub>2</sub>(OH)<sub>2</sub> precipitates were used as the standards for U(IV) and U(VI), respectively. Data on the amount of U accumulated by *B. subtilis*, kaolinite, and the mixture were collected at beam line 27B in the Photon Factory of High Energy Research Organization (Tsukuba, Japan). Spectra were measured in

the fluorescence mode for all samples using a 7-elements Ge array detector interfaced to single-channel analyzers. To prevent the oxidation of U(IV) to U(VI) during transportation to the Photon Factory, the precipitates were placed in a sealed plastic bag that, in turn, was put into an outer plastic bag containing an oxygen absorbent. The outer bag made of material preventing the penetration of oxygen was removed just before making the XANES measurements.

The samples containing  $2.0 \text{ g L}^{-1}$  *B. subtilis* and  $38 \text{ g L}^{-1}$  kaolinite exposed to a  $4 \times 10^{-4}$  M U(VI) solution at pH 4.7 were observed by transmission electron microscopy (TEM) (JEOL JEM-2000FXII), operated at 200 kV under standard conditions. Unstained samples were prepared by drying diluted ( $\times 200$ ) aliquots of suspension on holey carbon films (a whole mount). We employed a NORAN EDS system (NORAN Series II), mounted on electron microscope and operated at 200 kV to semi-quantitatively identify the elements.

### 3. Results

#### 3.1. Adsorption and desorption of U by the mixtures of *B. subtilis* and kaolinite

Fig. 1 shows the accumulated fraction of U by a mixture of *B. subtilis* and kaolinite as a function of dry weight percent fraction of *B. subtilis*. Kaolinite adsorbed 45% of the uranium from a U solution of  $8 \times 10^{-6}$  M; the amount rose to 95% with an increase

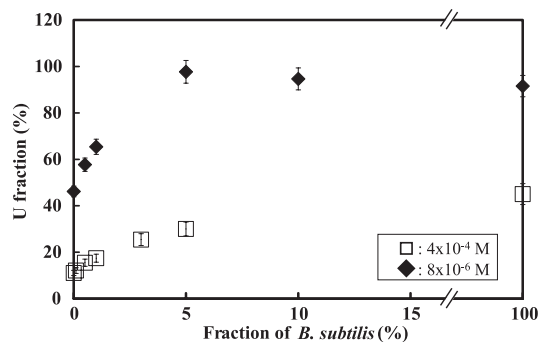


Fig. 1. Percent fractions of U accumulated by mixtures containing different amounts of *B. subtilis* and kaolinite from U(VI) solutions of  $8 \times 10^{-6}$  and  $4 \times 10^{-4}$  M. Bars show the range between high and low values.

in the fraction of *B. subtilis* up to 5%. In a solution containing of  $4 \times 10^{-4}$  M uranium, approximately 10% of U was removed by kaolinite, and about 40% by *B. subtilis*. Removal of uranium increased to approximately 30% with increasing the fraction of *B. subtilis* up to 5% in the mixtures.

Studies of the desorption of U by  $\text{CH}_3\text{COOK}$  from the  $8 \times 10^{-6}$  M U solution (Fig. 2a) indicated that approximately 80% of the accumulated U was desorbed from the kaolinite sample; approximately 55% was desorbed from *B. subtilis* sample, and a similar amount from the mixture containing 95% kaolinite and 5% *B. subtilis*. With U at  $4 \times 10^{-4}$  M, approximately 80% was desorbed from the sample containing kaolinite alone, while about 65% was removed from the sample containing 95% kaolinite and 5% *B. subtilis* and from the sample containing *B. subtilis* only

(Fig. 2b). The mean values of the fraction desorbed were found to be statistically different by using Student's *t*-test with a 95% confidence.

These results indicated that adding *B. subtilis* to the kaolinite slurry enhanced U accumulation. Furthermore, the higher the numbers of *B. subtilis* cells in the mixtures the lower was the desorption of U suggesting that U was more tightly bound. The sorption and desorption behaviors of U at  $8 \times 10^{-6}$  M resembled those at  $4 \times 10^{-4}$  M, even though the amounts of U sorbed and desorbed differed at different concentrations of U.

### 3.2. TEM observation and EDS analysis

Whole mounts of samples of the mixtures exposed to  $4 \times 10^{-4}$  M U(VI) solution showed clear

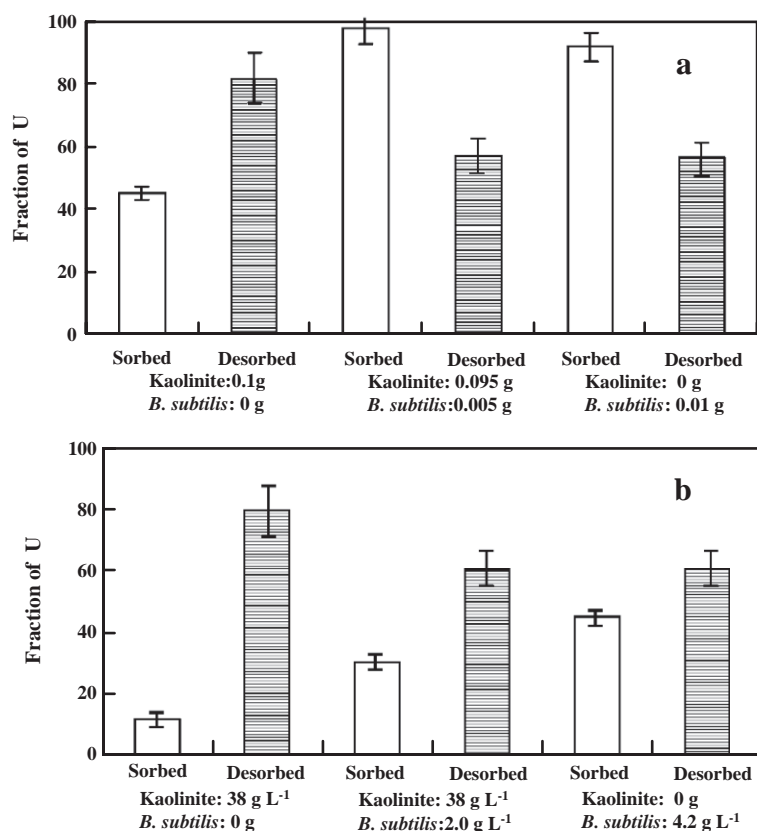


Fig. 2. Percent fractions of U of  $8 \times 10^{-6}$  M (a) and  $4 \times 10^{-4}$  M (b) adsorbed by, and desorbed with, a 1 M  $\text{CH}_3\text{COOK}$  solution of pH 4.7 from kaolinite of  $38 \text{ g L}^{-1}$ , the mixture containing kaolinite of  $38 \text{ g L}^{-1}$  and *B. subtilis* of  $2.0 \text{ g L}^{-1}$ , and *B. subtilis* of  $4.2 \text{ g L}^{-1}$ . The desorbed fraction of U was normalized to 100 when all of the sorbed U was removed with the  $\text{CH}_3\text{COOK}$ . Bars show the range between high and low values.

contrast in the TEM image without further staining (Fig. 3a). Neither the *B. subtilis* cells nor the kaolinite particles showed any evidence of a precipitate on their surfaces. The EDS spectrum of *B. subtilis* cells (Fig. 3b) showed a distinct peak of U M $\alpha$  and small peaks of Al K $\alpha$ , Si K $\alpha$ , P K $\alpha$ , and S K $\alpha$ . By contrast the EDS spectrum of the kaolinite particles revealed distinct peaks of Al K $\alpha$  and Si K $\alpha$  whereas no peak of U M $\alpha$  was detected. Similar EDS spectra were obtained for kaolinite and *B. subtilis* at the five different positions depicted in Fig. 3a. These results verified that almost all of the U was associated with the cells of *B. subtilis* in the mixture under the experimental conditions.

### 3.3. XANES analysis

Fig. 4 shows the averaged normalized XANES spectra of the U(IV) and U(VI) standards and the bacterium, the kaolinite, and the mixture after U

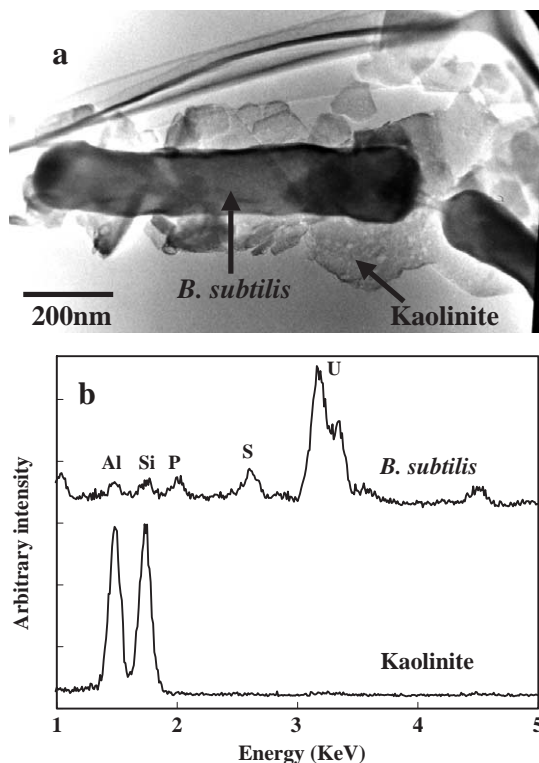


Fig. 3. TEM image of the U accumulated mixture containing *B. subtilis* and kaolinite mixture (a), and EDS spectra of *B. subtilis* and kaolinite (b).

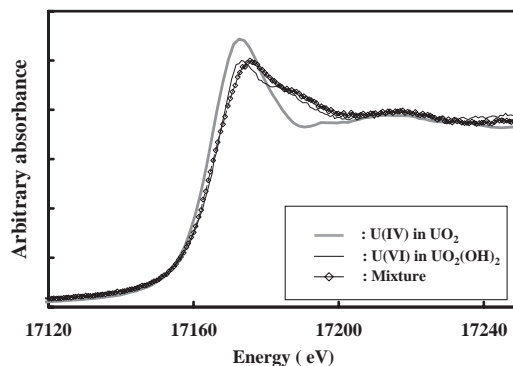


Fig. 4. XANES spectra of the U(IV) and U(VI) standards and the U-accumulated mixture. XANES spectra of the U-accumulated kaolinite and *B. subtilis* are not shown, but were nearly the same as that of the U-accumulated mixture.

accumulation; the spectra of the *B. subtilis* and kaolinite were the same as that of the U-accumulated mixture. The uranium L<sub>3</sub>-edge position of the mixture was consistent with the edge position of the U(VI) standard, indicating that the oxidation state of U in *B. subtilis*, kaolinite, and the mixture was U(VI). Thus, the preferential accumulation of U on to the bacteria in the mixtures under the experimental conditions could not be attributed to the reduction of U(VI) to U(IV) during exposure.

## 4. Discussion

TEM and EDS analyses of *B. subtilis* and kaolinite showed no evidence of precipitation of uranium on the surface. However, rod-shaped uranyl-phosphate minerals (Renninger et al., 2001) and uraninite particles (Suzuki et al., 2002) were observed around bacterial cells by TEM. The inconsistency between our results and those of Renninger et al. (2001) and Suzuki et al. (2002) suggests that U precipitation is not a dominant accumulation mechanism in the mixtures of *B. subtilis* and kaolinite under the experimental conditions. No uraninite formation is consistent with the XANES analysis that oxidation state of U is VI.

The sorption of Sr(II) has been reported for the mixture of bacteria and amorphous hydrous Fe-oxides (HFO) (Small et al., 1999). The sorption isotherm model analysis indirectly showed that Sr(II) was associated with bacteria and HFO in the mixture. The present results showed the direct evidence of the

preferential accumulation of U(VI) to the cell surface of *B. subtilis* in the mixture of *B. subtilis* and kaolinite clay. Thus, the accumulation behavior of U(VI) by the mixture of *B. subtilis* and kaolinite clay differs from that of Sr(II) on the mixture of bacteria and HFO.

The mode of accumulation of U(VI) could be due to sorption by the functional groups present in the cell surface of *B. subtilis* and on the edge sites of kaolinite by precipitation to form uranyl-containing minerals or by reduction to insoluble U(IV). Others reported that kaolinite accumulates U(VI) principally by sorption on edge sites (Turner and Sassman, 1996; Kohler et al. 1992). Our results indicate that the accumulation of U(VI) by the mixture was neither by reduction to U(IV) nor precipitation of an uranyl-containing mineral. Thus, adsorption of U(VI) on the surface of *B. subtilis* and kaolinite is the dominant mechanism in the accumulation of U(VI) by the mixtures. The preferential association of U(VI) with *B. subtilis* in the mixture of *B. subtilis* and kaolinite observed by TEM and EDS analyses (Fig. 3a and b) is probably due to differences between their mechanisms of U(VI) sorption.

Kelly et al. (2002) showed by X-ray absorption fine structure (XAFS) analysis that in *B. subtilis* U(VI) is bound to a protonated phosphoryl group at pHs below 3, but is increasingly bound to a deprotonated carboxyl functional groups with increasing pH. They suggested that fraction of U(VI) binding to a protonated phosphoryl group is constant at pHs between 1.7 and 4.8. These findings suggest that U(VI) is associated with both protonated phosphoryl and deprotonated carboxyl groups on the cell surface of *B. subtilis* under the experimental conditions. This association is in good agreement with the surface complex model study by Fowle et al. (2000) for *B. subtilis*.

Using surface complex modeling to determine the adsorption onto kaolinite, Turner and Sassman (1996) and Kohler et al. (1992) suggested that U(VI) is associated with silanol and aluminol groups at edge sites. Kohler et al. (1992) pointed out that U(VI) is mainly associated with the aluminol group. XAFS studies of U(VI) sorption onto silica gels and silicic acid indicated that uranyl undergoes inner-sphere surface complexation (Reich et al., 1996, 1998). Surface complexation and XAFS studies of U(VI) sorption onto montmorillonite suggested that the association

of U(VI) with silanol and aluminol groups rises with increasing pH.

These results suggest that U(VI) forms surface complexes with the functional groups of the cellular surface of *B. subtilis* and/or edge sites of kaolinite. Approximately 80% of the accumulated U was desorbed with the CH<sub>3</sub>COOK solution from kaolinite at both U concentrations, a result implying that the U bound by the silanol and aluminol groups is desorbable with CH<sub>3</sub>COOK. Our results agree with the report by Yanase et al. (1996) that more than 98% U(VI) was not adsorbed by the clay minerals of chlorite and smectite in a 1 M CH<sub>3</sub>COONa solution at pH 5.0 (Yanase et al., 1996). A much smaller fraction of the sorbed U(VI) was removed by CH<sub>3</sub>COOK from *B. subtilis* than from kaolinite (Fig. 2a and b) suggesting that U(VI) is more tightly associated with the functional groups on the surface of *B. subtilis* than those on the edge sites of kaolinite probably due to differences between their mechanisms sorption of U(VI). We assume that the U(VI) complex with the carboxyl group should have nearly the same stability constant as that with acetic acid because of the structural similarity of these functional groups. Accordingly this supposition leads to the idea that the U(VI) associated with carboxyl functional groups could be desorbed with CH<sub>3</sub>COOK. Therefore, the remaining fraction of U(VI) after desorption from *B. subtilis* with CH<sub>3</sub>COOK might be bound strongly to structures other than the carboxyl functional groups, i.e., phosphoryl groups on the surface of *B. subtilis*.

## 5. Conclusions

The preferential association of U(VI) with *B. subtilis* was observed by TEM and EDS analyses of a mixture consisting of the bacterium and kaolinite. Desorption of U(VI) with a 1 M CH<sub>3</sub>COOK solution from a mixture of *B. subtilis* and kaolinite indicated a tighter association with the former. Differences in the functional groups able to bind U(VI) cause the preferential association of U(VI) to *B. subtilis* rather than kaolinite in the mixtures under the experimental conditions. These results suggest that bacteria play an important role in regulating the mobility of U in the environment.

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## References

- Airey, P.L., Ivanovich, M., 1986. Geochemical analogues of high-level radioactive waste repositories. *Chem. Geol.* 55, 203–213.
- Brantley, S.L., Liermann, L., Bau, M., 2001. Uptake of trace metals and rare earth elements from hornblende by a soil bacterium. *Geomicrobiol. J.* 18, 37–61.
- Buck, E.C., Brown, N.R., Dietz, N.L., 1996. Contaminant uranium phases and leaching at the Fernald site in Ohio. *Environ. Sci. Technol.* 30, 81–88.
- Dent, A.J., Ramsay, J.D., Swanton, S.W., 1992. An EXAFS study of uranyl ion in solution and sorbed onto silica and montmorillonite clay colloids. *J. Colloid Interface Sci.* 150, 45–59.
- Fowle, D.A., Fein, J.B., Martin, A.M., 2000. Experimental study of uranyl adsorption onto *Bacillus subtilis*. *Environ. Sci. Technol.* 34, 3737–3741.
- Francis, A.J., Dodge, D.J., Lu, F., Halada, G., Clayton, C.R., 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp.. *Environ. Sci. Technol.* 28, 636–639.
- Francis, A.J., Gillow, J.B., Dodge, C.J., Harris, R., Beveridge, T.J., Papenguth, H.W., 2004. Uranium association with halophilic and non-halophilic bacteria and archaea. *Radiochim. Acta* 92, 481–488.
- Haas, J.H., Dichristina, T.J., Wade, R. Jr., 2001. Thermodynamics of U (VI) sorption onto *Shewanella putrefaciens*. *Chem. Geol.* 180, 33–54.
- Jeong, B.C., Hawes, C., Bonthron, K.M., Macaskie, L.E., 1997. Localization of enzymatically enhanced heavy metal accumulation *Citrobacter* sp. and metal accumulation in vitro by liposomes containing entrapped enzyme. *Microbiology* 43, 2497–2507.
- Kelly, S.D., Kemner, K.M., Fein, J.B., Fowle, D.A., Boyanov, M.I., Bunker, B.A., Yee, N., 2002. X-ray absorption fine structure determination of pH dependent U-bacterial cell wall interaction. *Geochim. Cosmochim. Acta* 66, 3855–3871.
- Kohler, M., Wieland, E., Leckie, J.O., 1992. Metal-ligand-surface interactions during sorption of uranyl and neptunyl on oxides and silicates. In: Kharaka, Y.K., Maest, A.S. (Eds.), *Water-Rock Interaction (VII)*. Balkema, Rotterdam, pp. 51–54.
- Langmuir, D., 1978. Uranium solution—minerals equilibria at low temperatures with applications to sedimentary ore deposits. *Geochim. Cosmochim. Acta* 42, 547–569.
- Lovley, D.R., Phillips, E.J.P., Gorby, Y.A., Landa, E.R., 1991. Microbial reduction of uranium. *Nature* 350, 413–416.
- Macaskie, L.E., Empson, R.M., Cheetham, A.K., Grey, C.P.A., Skarnuli, J., 1992. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymatically mediated growth of polycrystalline  $\text{HUO}_2\text{PO}_4$ . *Science* 257, 782–784.
- Reich, T., Moll, H., Denecke, A., Geipel, G., Bernhard, G., Nitsche, H., Allen, P.G., Bucher, J.J., Kaltsoyannis, N., Edelstein, N.M., Shuh, D.K., 1996. Characterization of Hydrated Uranyl Silicate by EXAFS. *Radiochim. Acta* 74, 219–223.
- Reich, T., Moll, H., Arnold, T., Denecke, M.A., Henning, C., Geipel, G., Bernhard, G., Nitsche, H., Allen, P.G., Bucher, J.J., Edelstein, N.M., Shur, D.K., 1998. An EXAFS study of uranium (VI) sorption onto silica gel and ferrihydrite. *J. Elec. Spec. Rel. Phen.* 96, 237–243.
- Renninger, N., McMahan, K.D., Knopp, R., Nitsche, H., Clark, D.S., Keasling, J.D., 2001. Uranyl precipitation by biomass from an enhanced biological phosphorus removal reactor. *Bio-degradation* 12, 401–410.
- Small, T.D., Warren, L.A., Roden, E.E., Ferris, F.G., 1999. Sorption of strontium by bacteria, Fe(III) oxide, and bacteria-Fe(III) oxide composites. *Environ. Sci. Technol.* 33, 4465–4470.
- Suzuki, Y., Banfield, J.F., 1999. Geomicrobiology of uranium. In: Burns, P.C., Finch, R. (Eds.), *Uranium: Mineralogy, Geochemistry and the Environment*, Review in Mineralogy, vol. 38. Mineralogical Society of America, Washington, DC, pp. 393–432.
- Suzuki, Y., Kelly, S.D., Kemner, K.M., Banfield, J.F., 2002. Nanometer-size products of uranium bioreduction. *Nature* 419, 134.
- Sylwester, E.R., Hudson, E.A., Allen, P.G., 2000. The structure of uranium (VI) sorption complexes on silica, alumina, and montmorillonite. *Geochim. Cosmochim. Acta* 64, 2431–2438.
- Ticknor, K.V., 1994. Uranium sorption on geological materials. *Radiochim. Acta* 64, 229–236.
- Turner, D.R., Sassman, S.A., 1996. Approaches to sorption modeling for high-level waste performance assessment. *J. Contam. Hydrol.* 21, 311–332.
- Waite, T.D., Davis, J.A., Payne, T.E., Waychunas, G.A., Xu, N., 1994. Uranium(VI) adsorption to ferrihydrite: application of a surface complexation model. *Geochim. Cosmochim. Acta* 58, 5465–5478.
- Yanase, N., Sato, T., Isobe, T., Sekine, K., 1996. Selective extraction procedure and its importance in a natural analogue study—case study at the Koongarra uranium deposit. *Waste Manage. Res.* 2, 121–135.
- Young, P., Macaskie, L.E., 1995. Role of citrate as complexing ligand which permits enzymatically-mediated uranyl ion bioaccumulation. *Bull. Environ. Contam. Toxicol.* 54, 892–899.